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ORIGINAL ARTICLE

Resting heart rate variability and plasma noradrenaline level as a measurement of autonomic nervous system activity in mature, aging rats

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Abstract

Introduction and objective. Aging is a process that also affects the autonomic nervous system (ANS) making it less adaptable to environmental and intrinsic stimuli and affecting its ability to maintain body homeostasis. The aim of this study was to estimate the resting ANS function using heart rate variability (HRV) method and by noradrenaline measurement in aging, 2–12-months-old rats.

Materials and method. Resting 15-minute-long ECG recordings were performed in anaesthetized rats with a subsequent spectral HRV analysis. Basic non-normalized HRV components in the range of very low (VLF), low (LF) and high (HF) frequency, along with the total HRV spectrum power (TP) were estimated. Moreover, normalized LF (nLF) and normalized HF (nHF) were calculated. Blood samples were also collected to assay plasma noradrenaline (NA) level.

Results. In the overall assessment, plasma noradrenaline level as well as both TP and all non-normalized HRV components demonstrated a tendency for reduction when compared the first (2^{nd}) and last (12^{th}) months. In the case of nLF and nHF, a trend of nLF predominance in the 2^{nd} and 3^{rd} month was revealed while an inverse relation was observed from the 6^{th} month on, with nHF superiority. Overall, males reached comparable or slightly higher NA and non-normalized HRV values compared to females, although most differences were not statistically significant. A parallel decline of LF (starting from the 10^{th} month) and HF (from the 6^{th} month) was demonstrated in both male and female animals. Female rats had a little more stable nLF and nHF course in the study time.

Conclusions. Rat ANS aging is associated with global HRV decrease with parallel plasma NA decline, although without selective impairment of individual (sympathetic/parasympathetic) ANS components.

Key words

autonomic nervous system, heart rate variability, aging, rat

INTRODUCTION

The autonomic nervous system (ANS) is essential for fast and adequate adaptation of visceral function in response to the continuously changing external and internal environment (maintenance of homeostasis).

Functionally, ANS is immature at birth and undergoes postnatal maturation. There is a general belief that the parasympathetic ANS branch is more developed compared to the sympathetic one at birth. The latter is thought to reach its full activity after several months of postnatal life, depending on the species (including humans) [1]. That statement is based on both histochemical and physiological data. The histochemical marker for sympathetic innervation – tyrosine hydroxylase – increases progressively in canine heart until 2 months after birth [2]. Human immunohistochemical studies also revealed that the density of cardiac sympathetic innervation (indicated by tyrosine hydroxylase and dopamine beta-hydroxylase) is significantly less in the newborn heart than in the adult one [3].

The whole period after completion of the maturation process may be trivially referred to as a progressive ageing process. Thus, ageing is a gradual, continuous process of changes continuing throughout the mature life. Ageing or senescence can be considered as a sum of losses of both functions and morphological structures. That process also affects the ANS [4]. The indirect evidence supporting the thesis of overall decline in the ANS function with age is the progressive, age-dependent function deterioration of systems under autonomic control. Therefore, the incidence of numerous diseases increases with age and, on the other hand, the course of many chronic diseases becomes deteriorated by autonomic nervous system dysfunction [5]. Alterations affecting both the central and peripheral neuronal structures, being parts of both the somatic and the autonomic nervous system, include loss of the total neuronal count and reconstruction of remaining neurons (decrease of axons, disturbed myelination). The decrease of melanin and dopamine (especially in substantia nigra) along with the decline in many other neurotransmitter levels have been also reported. Another important issue is the loss of numerous neuronal receptors and their diminished affinity to their specific agonists [5, 6].

There are many studies evaluating the impaired autonomic nervous system function in various conditions, primarily based on various clinical, dynamic methods of the ANS assessment (e.g. using so-called autonomic tests – intentional induction and assessment of certain cardiovascular reflexes).

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Although aging (including ANS senescence) is an obvious physiological process, it remains poorly understood. Clinical reports providing a detailed characterization of age-related functional ANS changes are scant. Moreover, there are no experimental research programmes devoted to evaluation of resting activity of the aging ANS.

This is partly due to technical difficulties – in practice, there is no direct assessment of the autonomic nervous system activity using some reliable laboratory techniques or direct neurophysiological records; and available, indirect methods are based on the assessment of autonomically-controlled effects. There are some possibilities of functional ANS assessment presented in numerous reviews [7–10], including our previously published paper [11]. One of the most widely used and readily available method of both clinical and experimental ANS activity assessment is the heart rate variability (HRV) [12]. This refers to the beat-to-beat (N-N; 'normal-normal') variation in the heart rate recorded in the ECG, resulting from the interplay of the sympathetic and parasympathetic modulation of the sinus node of the heart.

In addition, plasma noradrenaline measurement is regarded to be a laboratory surrogate marker of the sympathetic activity [11].

OBJECTIVES

The study aimed to estimate changes in the resting spectral heart rate variability, and the plasma noradrenaline level in anaesthetized rats throughout 2–12 months of life. The zootechnical data indicate that rat reaches sexual maturity 5–8 weeks after birth, and full housing maturity in about 8–10 weeks, while the rat's average life expectancy is about 2 years [13,14]. Considering these data, it could be stated that animals ending the puberty period (2 months-old) and completely mature (3–12 months-old), already aging, were studied.

MATERIAL AND METHODS

The study was approved by the First Local Ethical Committee for Animal Experiments in Krakow.

General plan of the experiment. The study was carried out on laboratory Wistar rats, 2–12 months-old, obtained from the central animal house of the Faculty of Pharmacy UJCM in Krakow. Before reaching the desired age, animals were kept in the above-mentioned research unit and were subsequently transferred to a local animal house of the Department of Pathophysiology UJCM. Upon arrival, animals acclimatized to new living conditions for 10 days. Rats were housed in unisex groups and depending on their age and weight, they were kept in groups of 2–3 individuals in standard laboratory cages, with unlimited access to water and laboratory chow (Biowet, Pulawy), with the addition of 'enrichments' to ensure complete animal welfare. During that period, rats were subject to daily observation and handling, allowing confirmation of their good health and physiological state.

After the acclimatization period, general anesthesia was induced in individual animals to perform the standardized ECG records followed by the HRV analysis.

Finally, after administration of a dose of sodium pentobarbital(400 mg/kg b.w.), blood samples were collected from each animal. Plasma was separated and stored at -70 °C for further biochemical assay of noradrenaline concentration.

Studied animals. 2, 3, 4, 6, 8, 10 and 12 months-old rats, both males (M) and females (F) rats were enrolled. Depending on the gender and age of the animals, 14 groups of 6 animals each were examined (mean body weight [g] ± SEM in each study group was given in the parentheses below): M2M (male; 2 months; 281.8±12.6), F2M (female; 2 months; 196.3±15.6), M3M (male; 3months; 282.9±12.0), F3M (female; 3 months; 207.0±26.0), M4M (male; 4 months; 359.4±38.3), F4M (female; 4 months; 211.3±21.7), M6M (male; 6 months; 450.8±59.6), F6M (female; 6 months; 248.8±16.3), M8M (male; 8 months; 460.3±52.0), F8M (female; 8 months; 243.2±10.4), M10M (male, 10 months; 479.2±38.6), F10M (female; 10 months; 287.3±11.4), and M12M (male, 12 months; 516.0±26.9), F12M (female; 12 months; 276.5±21.1). The total number of animals used in the experiment was 84.

ECG recordings and HRV analysis methods. The ECG was recorded under general pentobarbital sodium-induced anaesthesia (Morbital, 50 mg per kg body weight, i.p.) and 20-min rest. Before the recordings, the abdominal fur was removed, rats' abdomens were carefully shaved in order to achieve epidermal abrasion and a standard ECG gel was applied. Recordings were collected with paediatric Ag/AgCl probes (EG-S30 PSG Sorimex), deployed in the classic configuration in order to obtain one ECG lead, and the ADInstruments hardware (Power Lab 4/30 and BIO Amplifier). The fragment, including the first 5 minutes, was excluded from subsequent analysis and was regarded as the time necessary for signal calibration and adaptation of the study individual to new experimental conditions. During the registration, rats were placed under a heating lamp to protect them from body temperature drop.

Once registrations were completed, the 15-minute-long ECG signal was visually evaluated to remove ectopic beats, and the remaining records were subject to the HRV analysis, using the ADInstruments software (Chart v5.4.2) for Mac OS X Version 10.1.2. According to the HRV guidelines, both time- and spectral domain analysis are recommended for long-lasting (especially 24-hour) ECG recordings, while short ones (5–30 minutes) should be subjected only to the spectral (frequency) analysis [12].

HRV analysis is based on the variability of duration of adjacent, 'normal-normal' (N-N) intervals, subject to continuous, ANS-modulated fluctuations. The spectral (frequency) HRV analysis results from subjecting of the N-N intervals variability to fast-Fourier transformation or autoregression methods. In that procedure, the total power(TP) of HRV spectrum is determined along with the powers (in [ms^2]) of its basic components, resulting from distribution of RR intervals variability in relation to cyclic, ANS-modulated stimulating activity of the sinus node associated with 3 principal rhythms: very low frequency (VLF), low frequency (LF), and high frequency (HF) [12].

In clinical studies, ranges for the individual spectral components are: 0.003<VLF<0.04<LF<0.15<HF<0.4. Taking into account a rat's heart rate (much higher than in humans), the following ranges were adopted for individual HRV spectral components for the spectral HRV

analysis: 0.18<VLF<0.28<LF<0.78<HF<3. The frequency ranges adopted were similar to those used by Aubert et al. [15] (0.19<LF<0.74<HF<2.5) and Goncalves et al. [16] (0.10<LF<1.0<HF<3.0).

Total HRV spectrum power is said to reflect the global autonomic activity. Spectral HRV components are considered to be correlated with physiological functions: LF is regarded to be a marker of both sympathetic and vagal tension, also reflecting baroreflex sensitivity, while HF is agreed to be a selective, vagal –dependent HRV spectrum component. The VLF origin, although it constitutes the majority of the HRV spectrum, is the most controversial one, probably resulting from thermoregulatory and neuroimmunological processes and the cyclic activation of various regulatory mechanisms (e.g. renin-angiotensin-aldosterone system) [7–10].

Subjecting the HRV spectrum to the process of normalization, we also calculated the normalized nHF and nLF values, that are regarded to reflect the selective parasympathetic and sympathetic tension, respectively. The HRV spectrum normalization is based on the calculation of the share of respective components (LF or HF) in the total HRV power, excluding the VLF component power. That procedure results from difficulties in interpretation of the VLF component [8–11].

Noradrenaline assessment: Rat noradrenaline was measured in plasma samples using a commercially available ELISA kit (LDN; Labor Diagnostica Nord, GmbH & Co. KG; Nordhorn, Germany), strictly according to the manufacturer's instructions. Noradrenaline concentrations (ng/ml) in appropriate groups and time points (along with the total estimation) were assessed.

Statistical analysis. Since the calculated HRV parameters were characterized by large intragroup differences, manifested by high values of standard deviations, the output HRV parameters were subjected to logarithmic forms to ensure better approximation to normal distribution. The analysis estimating age and gender-dependent difference was performed using paired Student's t-test, separately for each paired (male-female) groups for each study month.The statistical significance level was set as $p \leq 0.05$.

RESULTS

Overall assessment

Noradrenaline. In the 2nd and 3rd month, comparable NA concentrations were noted. From the 4th month on, a continuous decrease was observed which was particularly pronounced in the last (10th and 12th) months of the experiment.

HRV. In the case of both TP and VLF, a progressive, continuous increase was noted in the 2nd and 3rd months, with a plateau in the 4th month,and with a subsequent decrease in the following months.

Considering LF and HF changes, it was demonstrated that HF reached higher values compared to LF in all study months, except for the last, 12th month, when LF achieved a slightly larger value. Analyzing the LF and HF changes in time, a stable course or progressive decline was noted, except for a transient LF and HF increase in the 6th month.

In case of nLF and nHF, a trend of nLF predominance in the 2nd and 3rd months was observed, while from the 6th month on, an inverse relation was observed, with nHF superiority. In the 4th month, there was a perfect balance between nLF and nHF.

Detailed results of the logarithmic values of both NA concentrations and spectral HRV parameters in studied rats, without gender differentiation, are given in (Table 1).

Gender and age dependent HRV and NA differences. Detailed results (expressed in logarithmic values of NA concentration and HRV parameters), revealed separately in male and female animals, along with the statistical reasoning, are presented in Table 2.

Noradrenaline. Considering the NA differences, almost the same plasma NA levels were observed in the 2nd, 3rd and 4th months,while from the 6th month on, some significantly higher values were observed in males. On the other hand, in the 12th month, a slightly higher value was demonstrated for female rats.

The trend of the course of the plasma NA changes during the study months is presented in the Figure 1 (graph represents mean values calculated for studied months). Moreover, the curve, created by connecting points determined in studied months, also demonstrates some hypothetical values for those months in which no HRV assessment was performed.

HRV. Male animals had higher TP values comparing to females in the first 6 study months. In the 8th month, the relationship became inverse, and starting from and 10th month the male predominance was observed again. In general, the courses of TP decline, were almost parallel in male and female rats, except for the 8th month. VLF changes were more irregular, especially those observed during months 6–12.

Table 1. Logarithmic plasma noradrenaline (NA) concentrations (mean±SD), spectral HRV parameters (mean±SD) and resting heart rate (HR; mean±SD) in individual months of the experiment – overall assessment (for both males and females)

	NA [ln ng/ml]	TP [ln ms²]	VLF [ln ms²]	LF [ln ms²]	HF [ln ms²]	nLF [ln n.u.]	nHF [ln n.u.]	HR [1/min]
2M	2.82±0.11	3.79±0.66	3.15±0.57	2.00±0.94	2.37±0.99	4.04±0.17	3.71±0.28	374.54±26.75
3M	2.86±0.17	4.12±0.69	3.62±0.89	2.08±0.69	2.14±1.09	3.90±0.57	3.77±0.29	349.33±18.72
4M	2.37±0.57	4.14±0.35	3.64±0.57	2.09±0.65	2.11±0.89	3.88±0.32	3.88±0.22	368.35±26.09
6M	2.28±0.47	3.83±1.34	3.41±1.28	2.42±0.37	2.52±0.87	3.79±0.48	3.89±0.33	350.65±14.79
8M	2.01±0.52	3.06±1.15	2.68±1.00	0.81±1.29	1.31±1.71	3.54±0.64	4.04±0.35	357.29±19.94
10M	0.82±0.78	3.34 ±0.68	2.80±0.79	1.21±1.19	1.40±1.32	3.79±0.26	3.98±0.21	357.63±19.48
12M	-0.59±0.55	2.53±0.99	2.32±1.16	-0.31±0.97	-0.21±0.63	3.76±0.64	3.88±0.34	344.29±16.97

Table 2. Results and statistical analysis of logarithmic NA values (mean ± SD) and spectral HRV parameters (mean ± SD) in male and female rats in individual months of the experiment

		NA [ng/ml]	p	TP [ln ms^2]	p	VLF [ln ms^2]	p	LF [ln ms^2]	p	HF [ln ms^2]	p	nLF [ln n.u.]	p	nHF [ln n.u.]	p
2M	Male	2.79±0.13	0.27	3.99±0.52	0.21	3.13±0.23	0.46	2.23±1.21	0.30	2.88±0.88	0.09	4.17±0.14	**0.02**	3.51±0.30	**0.05**
	Female	2.85±0.10		3.63±0.77		3.17±0.78		1.83±0.77		1.97±0.97		3.95±0.13		3.86±0.15	
3M	Male	2.81±0.12	0.21	4.19±0.46	0.24	3.70±0.68	0.35	2.26±0.51	0.23	1.92±1.46	0.31	3.76±0.66	0.15	3.87±0.36	0.22
	Female	2.91±0.21		3.80±0.93		3.45±1.08		1.85±0.90		1.56±0.91		4.05±0.05		3.75±0.07	
4M	Male	2.32±0.67	0.40	4.27±0.08	0.10	3.60±0.65	0.41	2.47±0.18	**0.05**	2.64±0.78	**0.02**	3.78±0.44	0.17	3.95±0.27	0.19
	Female	2.41±0.51		3.96±0.45		3.68±0.56		1.74±0.77		1.55±0.67		3.99±0.11		3.81±0.15	
6M	Male	2.32±0.55	0.26	4.30±0.15	0.19	3.82±0.56	0.20	2.31±0.32	0.15	2.45±0.82	0.36	3.79±0.43	0.30	3.94±0.26	0.38
	Female	2.16±0.03		3.09±1.31		2.64±1.35		1.22±1.78		1.45±1.75		3.61±0.57		4.01±0.41	
8M	Male	2.42±0.24	**0.001**	3.29±0.86	**0.05**	2.13±1.13	0.08	0.96±0.80	**0.04**	1.86±1.29	0.44	3.30±0.67	0.14	4.20±0.24	0.14
	Female	1.60±0.34		4.07±0.14		3.43±0.76		1.90±0.55		1.92±1.54		3.82±0.56		3.85±0.42	
10M	Male	1.63±0.31	**0.001**	3.60±0.77	0.08	3.27±0.80	**0.01**	0.94±1.36	0.38	1.30±1.64	0.39	3.68±0.32	0.07	4.04±0.28	0.16
	Female	0.29±0.42		3.02±0.44		2.23±0.12		1.54±1.01		1.53±0.99		3.91±0.07		3.91±0.07	
12M	Male	0.48±0.74	0.33	2.88±0.87	0.15	2.76±0.96	0.13	-0.44±0.70	0.36	-0.33±0.80	0.27	3.75±0.77	0.48	3.85±0.37	0.39
	Female	0.32±0.42		2.10±1.01		1.78±1.29		-0.16±1.20		-0.06±0.50		3.77±0.55		3.91±0.33	

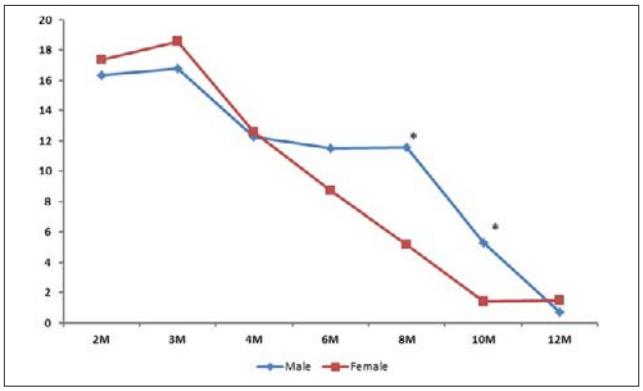

Figure 1. Trend of mean noradrenaline concentration [ng/ml] changes during the experiment in male and female rats
* p<0.05

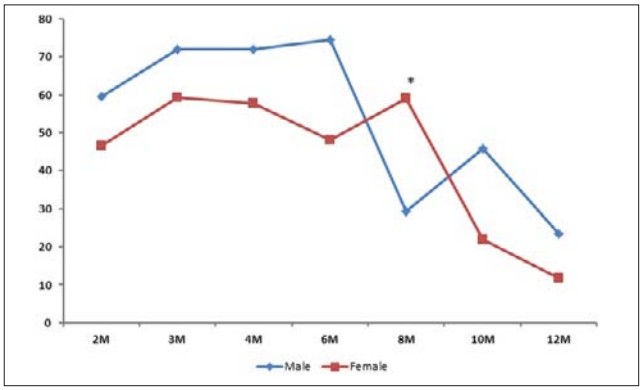

Figure 2. Trend of mean total power (TP) [ms^2] changes during the experiment in male and female rats
* p<0.05

In the LF analysis, higher values for male rats were demonstrated in months 2–6 (however, the difference was statistically significant only in the 4th month). Starting from the 8th month, an inverse, statistically significant difference was observed, with a higher LF power for female animals. Starting from the 10th month, almost the same, declined LF values were observed in both male and female animals. The similar course of the power changes in time was observed in the case of the HF component, but from the 8th month on, both male and female rats reached almost the same, parallel values.

For both normalized components, nLF and nHF, some more irregular changes were observed in following months, which were more marked in males. Female rats demonstrated some gentle, more linear nLF/nHF changes in time.

The detailed illustration of the trend of changes of the basic and the normalized spectral HRV components are given in Figures 2–7 (each of the graphs represents the mean values for each month). Moreover, the curve, created by connecting points for individual months, demonstrates also hypothetical values for those months in which no HRV assessment was performed.

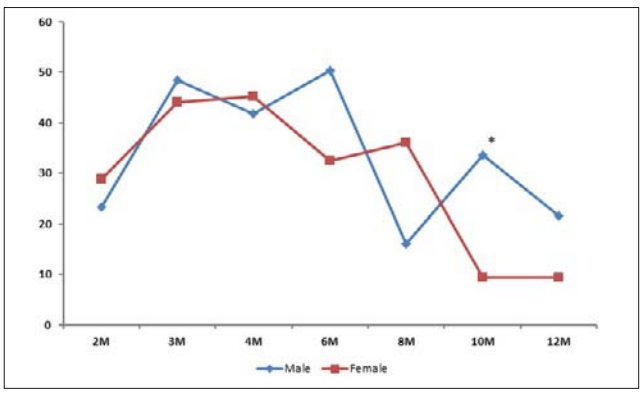

Figure 3. Trend of mean very low frequency (VLF) power [ms^2] changes during the experiment in male and female rats
* p<0.05

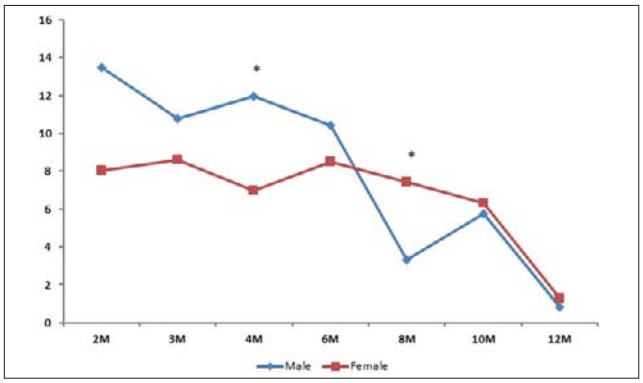

Figure 4. Trend of mean low frequency (LF) power [ms²] changes during the experiment in male and female rats
*p<0.05

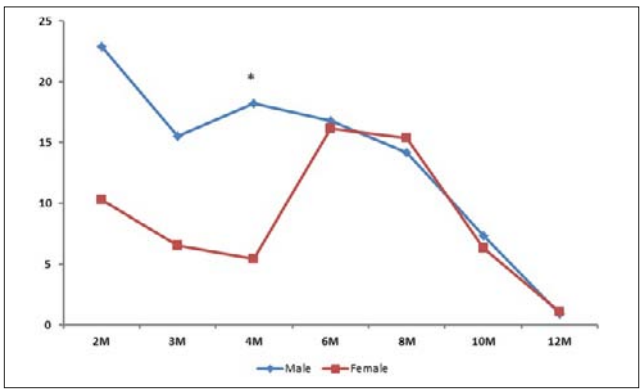

Figure 5. Trend of mean high frequency (HF) power [ms²] changes during the experiment in male and female rats
* p<0.05

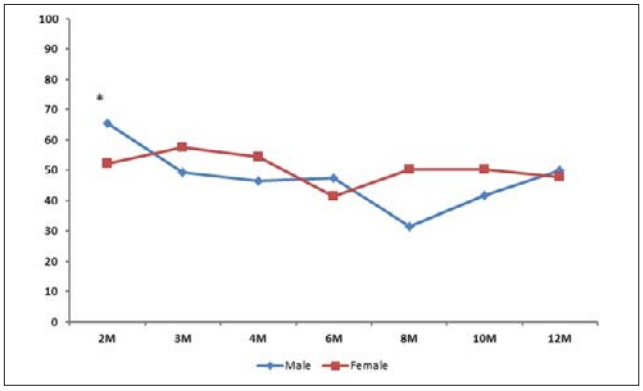

Figure 6. Trend of mean normalized low frequency (nLF) power [n.u.] changes during the experiment in male and female rats
*p<0.05

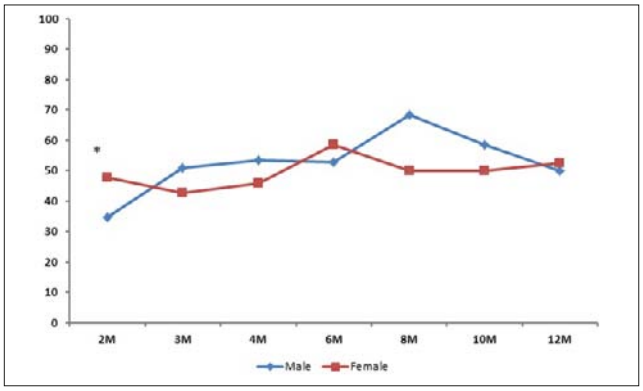

Figure 7. Trend of mean normalized high frequency (nHF) power [n.u.] changes during the experiment in male and female rats
* p<0.05

DISCUSSION

To sum up, the presented study confirmed that aging in rats is associated with the global HRV decrease. However, in resting HRV recordings obtained from anaesthetized rats, no preferential, aging-related parasympathetic or sympathetic impairment could be demonstrated.

According to the authors' knowledge, this is the first experimental study focusing on the assessment of resting changes in the ANS activity assessed by heart rate variability in mature, intact, aging rats.

As already mentioned in the Introduction, body function deteriorates with age and that impairment involves also the autonomic nervous system function. Therefore, the autonomic modulation of circulation, metabolism and digestion processes, resulting from the sympathetic and parasympathetic dysregulation, impairs the internal homeostasis [17].

Clinical studies also demonstrated that aging was associated with depressed HRV. In the 24-hour Holter recordings, Reardon et al. [18] proved that aging reduced the global HRV measure and concluded that the process may reflect a reduced responsiveness of autonomic activity to various stimuli with age. In another study performed by Akhter et al. [19], cardiac autonomic impairment with the advancement of age was demonstrated, and the detailed HRV analysis demonstrated a decreased parasympathetic function. Those findings were confirmed by Shankar and Veeraiah [20] who demonstrated a parasympathetic dysfunction in the elderly, using classical Ewing's clinical autonomic tests (deep breathing, Valsalva maneuver, standing orthostatic response). Therefore, the clinical thesis of progressive, global ANS tension reduction with the particular withdrawal of the parasympathetic activity is well documented in humans. Besides, it should be also mentioned that taking into account that the reduced share of the vagus nerve in the regulation of the heart rate is an unfavourable prognosis associated with an increased risk of serious cardiovascular events in people of advanced age, HRV analysis can be a useful, non-invasive tool for assessment of the function of the cardiovascular system in those patients [21]

There are also studies investigating both age and gender-dependent HRV changes in healthy subjects, e.g. Yukishita et al. [17] studied healthy volunteers in their 20s, 30s and 40+. In the analysis of 3-minute-long, resting ECG recordings the authors demonstrated that all HRV parameters were significantly negatively correlated with age in both men and women. Moreover, younger men (20s and 30s) demonstrated higher values of HRV parameters while HRV parameters reached higher values in 40+ women.

To sum up, clinical studies demonstrated a decline of the global autonomic activity with age, with marked parasympathetic dysfunction, more pronounced in elderly male subjects.

The HRV results in the presented study are partly consistent with those mentioned above, although it is obvious that one should not uncritically compare experimental and clinical results. However, the HRV spectrum in healthy rats resembles the human one. It also consists of 3 principal components: VLF, LF and HF, although due to the significantly higher heart and respiration rates, compared to those occurring in humans, the adopted rats' frequency ranges for individual HRV spectral components are different. In ECG recordings

obtained with a telemetric system from conscious rats, the HF peak appeared usually between 1 – 2.5 Hz (in accordance with the vagally-mediated, respiratory frequency in the rat) while the LF was detected at 0.2– 0.8 Hz [15, 22]. VLF band is often set up at about to 0.2 Hz [24]. These experimental data demonstrate that atropine administration significantly reduced HF while LF power decreased after the therapy with propranolol in rats [22, 24]. Therefore, in the analysis of the HRV in rats, with an acceptable approximation, one can adopt a similar interpretation assumptions as those validated in humans.

Thus, compared to clinical data, the current study also demonstrated a progressive reduction of overall autonomic activity (TP with NA concentration decreases) throughout the period between month 2 – 12. Also, considering the segmental LF decline, and the HF decline being more continuous for the final months of the study, the theory of a progressive, functional trend of both sympathetic and parasympathetic decrease with age could be stated. However, there is a discrepancy related to the analysis of normalized spectral components. Starting from the 6th month, in the overall analysis the nHF values were higher than nLF. Also, HF values were higher than LF in each month of the study, except for the last, 12th month. Therefore, in contrast to clinical data, the current study has not demonstrated any selective parasympathetic withdrawal in older age groups of animals.

Limitations of the study. The performed HRV analysis in rats adequately reflects the overall direction of physiological changes observed during the aging of the human ANS (but without revealing preferential impaired parasympathetic activity with age). Hence, that model may be applied to studies on the ANS aging assessing the overall changes of ANS activity. Moreover, it should be remembered, that those changes merely reflect the resting ANS activity and do not allow assessment of the dynamic autonomic response to any stimulation. In addition, even though rats are often used for the ANS studies, the key issues limiting widespread HRV studies in rats involve inadequate reproducibility of data related to difficulties in maintaining stable experimental conditions and a high behavioural sensitivity to environmental changes [23].

Moreover, this research was performed in anaesthetized rats and many available, published experimental studies, had been performed on conscious but restrained animals (conscious unrestrained animal studies are scare). Under anesthesia, the heart rate fluctuates, being a basis for its variability, are also influenced; therefore, HRV should be recorded in a precisely induced, shallow anesthesia [24, 25]. On the other hand, however, ECG in anesthetized rats is a compromise between the technical possibility of obtaining results of the HRV in general and the inevitable influence of the anesthetic agent on the obtained results. Similar objections are related to the ECG registration in conscious but restrained animals – the stress associated with immobilization also undoubtedly affect obtained HRV results.

Moreover, the influence of pentobarbital on the heart rate that is the background for subsequent heart rate variability is still open and a disputed issue. Literature data suggest that pentobarbital anesthesia in rats impairs baroreceptor activity and, consequently, results in a reduction of tachycardia, and a fall in blood pressure [26, 27]. However, according to some authors, the effect of pentobarbital on the resting heart rate (HR) in rats is slight, and it manifests by a daily modulation of heart rate (day-night differences) rather than by evoking of marked bradycardia after administration of single dose of that agent [28]. Therefore, according to Svorc et al. [28], the ECG registration (with subsequent HRV analysis) within the daylight period in rats anesthetized with pentobarbital, is acceptable if HR still remains within the reference for this species (250–500 / min) [14] because pentobarbital-mediated bradycardia resulted from vagal stimulation, is compensated by a parallel increase of the sympathetic activity. In the presented experiment, the heart rate of all studied rats in all the studied months remained within the normal range.

CONCLUSIONS

The main finding of this study is the demonstration that, in general, all non-normalized HRV parameters and the HRV total power decline with age, in both overall assessment and in gender-dependent estimation. Additionally, the reduced HRV was accompanied by a progressive decline in serum levels of noradrenaline. In general, male rats achieved comparable or higher HRV parameter values compared to female animals. On the other hand, in the case of females, a lower imbalances between sympathetic (nLF) and parasympathetic (nHF) tension in the course of study months was demonstrated, compared to males in which nLF-nHF changes were more irregular. A generalized reduction of ANS activity was not associated with selective functional ANS rebuilding, with selective impairment of one ANS branch.

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